Chapter 16

The Molecular Basis of Inheritance

Key Concepts

16.1 DNA is the genetic material

16.2 Many proteins work together in DNA replication and repair

16.3 A chromosome consists of a DNA molecule packed together with proteins

Framework

This chapter outlines the key evidence that was gathered to establish DNA as the molecule of inheritance. Watson and Crick's double helix, with its rungs of specifically paired nitrogenous bases and twisting phosphate-sugar side ropes, provided the three-dimensional model that explained DNA's ability to encode a great variety of information and produce exact copies of itself through semiconservative replication. The replication of DNA is an extremely fast and accurate process involving many enzymes and proteins. Eukaryotic chromosomes are complexes of DNA and protein that exhibit varying levels of packing during the cell cycle.

Chapter Review

Deoxyribonucleic acid, or DNA, is the genetic material that is transmitted from one generation to the next and encodes the blueprints that direct and control the biochemical, anatomical, physiological, and behavioral traits of organisms. DNA is precisely copied in the process of **DNA replication**.

16.1 DNA is the genetic material

The Search for the Genetic Material: Scientific Inquiry Chromosomes were shown to carry hereditary information and to consist of proteins and DNA. Until the 1940s, proteins seemed the most likely candidate to be the genetic material. The role of DNA was established through work with microorganisms—bacteria and viruses.

In 1928, F. Griffith was working with two strains of *Streptococcus pneumoniae.* When he mixed the remains of heat-killed pathogenic bacteria with harmless bacteria, some bacteria were changed into disease-causing bacteria. These bacteria incorporated external genetic material in a process called **transformation**, which results in a change in genotype and phenotype. Scientists later determined that DNA was the molecule that transformed bacteria.

Viruses consist of DNA (or sometimes RNA) contained in a protein coat. They reproduce by infecting a cell and commandeering that cell's metabolic machinery. **Bacteriophages**, or **phages**, are viruses that infect bacteria. In 1952, A. Hershey and M. Chase showed that DNA was the genetic material of a phage known as T2 that infects the bacterium *Escherichia coli.*

INTERACTIVE QUESTION 16.1

Hershey and Chase devised an experiment using radioactive isotopes to determine whether it was a phage's DNA or protein that entered the bacteria and served as the genetic material of T2 phage.

a. How did they label phage protein?

b. How did they label phage DNA?

After infecting separate samples of *E. coli* with the differently labeled T2 cells, they blended and centrifuged the samples to isolate the bacterial cells from the lighter viral particles.

c. Where was the radioactivity found in the samples with labeled phage protein?

d. Where was the radioactivity found in the samples with labeled phage DNA?

e. What did Hershey and Chase conclude from these results?

In 1950, E. Chargaff noted that the percentages of the four nitrogenous bases in DNA were species specific. Chargaff also determined that the number of adenines and thymines was approximately equal, and the number of guanines and cytosines was also equal. The variation in base composition among species and the A=T and G=C properties of DNA became known as *Chargaff's rules.*

Building a Structural Model of DNA: Scientific Inquiry By the early 1950s, the arrangement of covalent bonds in a nucleic acid polymer was established, but the three-dimensional structure of DNA was yet to be determined.

In X-ray crystallography, an X-ray beam passed through a substance produces an X-ray diffraction photo with a pattern of spots that a crystallographer interprets as information about three-dimensional molecular structure. J. Watson saw an X-ray photo produced by R. Franklin that indicated the helical shape of DNA and that it consisted of two strands—thus the term **double helix**.

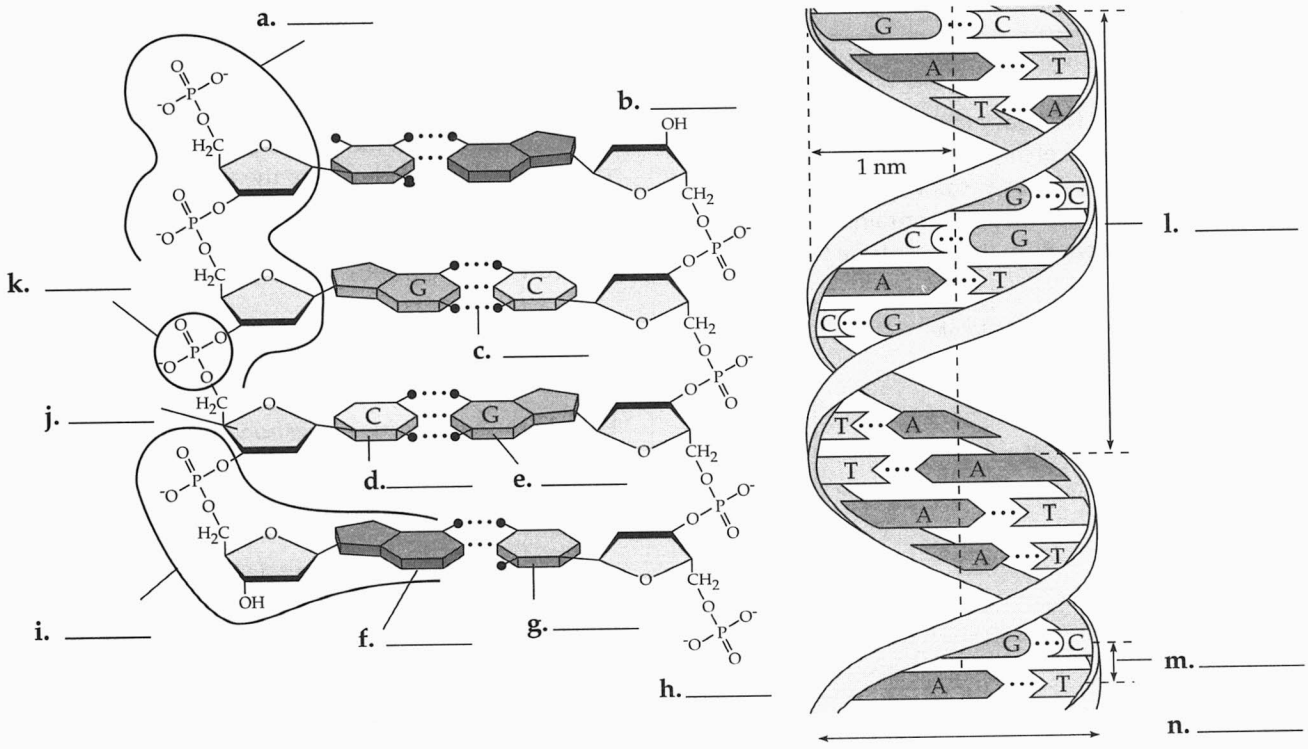
Watson and F. Crick constructed wire models of a double helix that had the paired nitrogenous bases on the inside of the helix and two sugar-phosphate chains running in opposite directions (**antiparallel**) on the outside. The helix makes one full turn every 3.4 nm; 10 layers of nucleotide pairs, stacked 0.34 nm apart, are present in each turn of the helix.

To produce the molecule's uniform 2-nm width, a purine base must pair with a pyrimidine base. The side groups of the bases permit two hydrogen bonds to form between adenine and thymine, and three hydrogen bonds between guanine and cytosine. This complementary pairing explains Chargaff's rules. Van der Waals attractions between the closely stacked bases help hold the molecule together.

In 1953, Watson and Crick published a paper in *Nature* reporting the double helix as the molecular model for DNA.

INTERACTIVE QUESTION 16.2

Review the structure of DNA by labeling the following diagrams.



16.2 Many proteins work together in DNA replication and repair

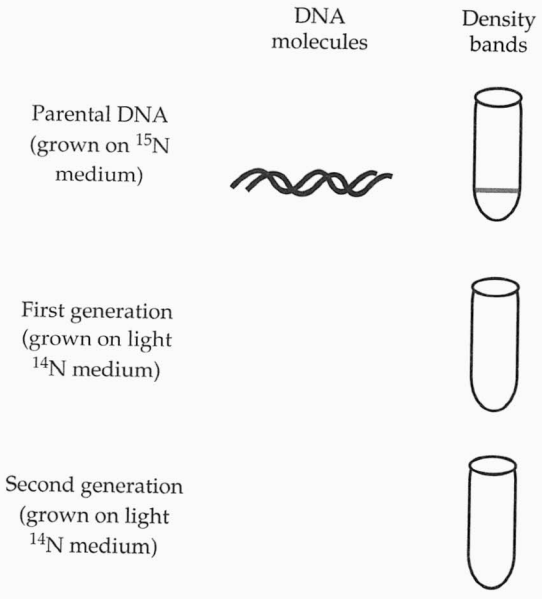
The Basic Principle: Base Pairing to a Template Strand Watson and Crick noted that the base-pairing rule of DNA sets up a mechanism for its replication. Each side of the double helix is an exact complement to the other. When the two sides separate, each strand serves as a template for rebuilding a double-stranded molecule identical to the "parental" molecule.

The **semiconservative model** of DNA replication predicts that the two daughter DNA molecules each have one parental strand and one newly formed strand. In contrast, a conservative model predicts that the parental double helix reforms and the duplicated molecule is totally new, whereas a dispersive model predicts that all four strands of the two DNA molecules are a mixture of parental and new DNA.

M. Meselson and F. Stahl tested these models by growing *E. coli* in a medium with ^{15}N, a heavy isotope that the bacteria incorporated into their nitrogenous bases. Cells with labeled DNA were transferred to a medium with a lighter isotope, ^{14}N. Samples were removed after one and two generations of bacterial growth, and DNA was extracted and centrifuged. The locations of the density bands in the centrifuge tubes confirmed the semiconservative model of DNA replication.

INTERACTIVE QUESTION 16.3

Using different colors for heavy (parental) and light (new) strands of DNA, sketch the DNA molecules formed in two replication cycles after *E. coli* were moved from medium containing ^{15}N to medium containing ^{14}N. Show the resulting density bands in the centrifuge tubes.



DNA Replication: A Closer Look Replication of most bacterial chromosomes begins at a single **origin of replication**, where proteins that initiate replication bind to a specific sequence of nucleotides and separate the two strands to form a replication "bubble." Replication proceeds in both directions in the two Y-shaped **replication forks**. Eukaryotic chromosomes have many origins of replication.

An enzyme called **helicase** unwinds the helix and separates the parental strands at each replication fork. **Single-strand binding proteins** keep the separated strands apart while they serve as templates. **Topoisomerase** helps relieve the strain from the tighter twisting of DNA strands in front of helicase. An enzyme called **primase** joins about 5 to 10 RNA nucleotides base-paired to the parental strand to form the **primer** needed to start the new DNA strand.

Enzymes called **DNA polymerases** connect nucleotides to the growing end of a new DNA strand. DNA polymerase III and I are involved in replication in *E. coli;* at least 11 different DNA polymerases have been discovered so far in eukaryotes. A nucleotide lines up with its complementary base on the template strand; it loses two phosphate groups, and the hydrolysis of this pyrophosphate to two inorganic phosphates ($\mathrm{P_i}$) provides the energy for polymerization.

Remember that the two strands of a DNA molecule are antiparallel—they are oriented in opposite directions. The sugar of each nucleotide is connected to its own phosphate group at its 5′ carbon and connects to the phosphate group of the adjacent nucleotide by its 3′ carbon. Thus, the polarity of a strand of DNA runs from the 5′ end, where the first nucleotide's phosphate group is exposed, to the 3′ end where the nucleotide at the other end has a hydroxyl group attached to its 3′ carbon.

INTERACTIVE QUESTION 16.4

Look back to Interactive Question 16.2 and label the 5′ and 3′ ends of the left strand of the DNA molecule.

DNA polymerases add nucleotides only to the free 3′ end of a primer or growing DNA strand; DNA is replicated in a 5′ → 3′ direction. The **leading strand** is the new continuous strand being formed along one template strand as DNA polymerase III (DNA pol III) remains in the progressing replication fork.

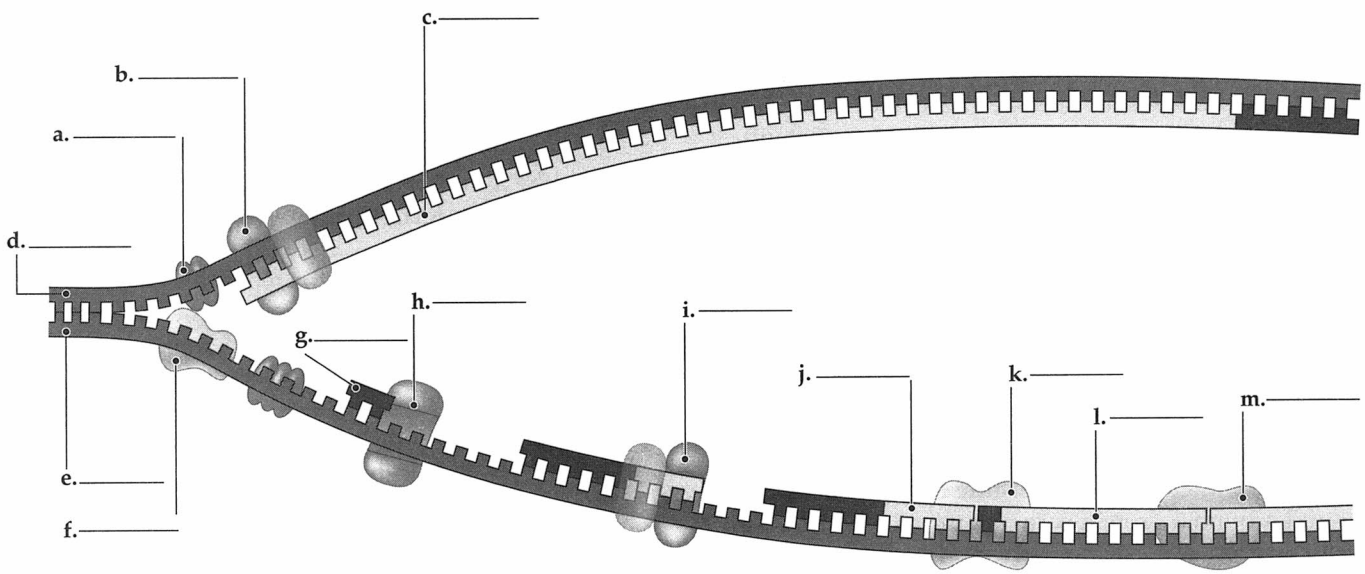
The **lagging strand** is created as a series of short segments, called **Okazaki fragments**, which are elongated in the 5′ → 3′ direction away from the replication fork. Each fragment on the lagging strand requires a primer. A continuous strand of DNA is

produced after both DNA polymerase I (DNA pol I) replaces the RNA primer with DNA nucleotides and an enzyme called **DNA ligase** joins the sugar-phosphate backbones of the fragments.

The various proteins that function in replication form a large DNA replication complex. In eukaryotic cells, many such complexes may anchor to the nuclear matrix, and the DNA polymerase molecules may pull the parental DNA strands through them.

INTERACTIVE QUESTION 16.5

In this diagram of bacterial DNA replication, label the following items: leading and lagging strands, Okazaki fragment, DNA pol III, DNA pol I, DNA ligase, helicase, single-strand binding proteins, primase, RNA primer, and 5′ and 3′ ends of parental DNA.



Proofreading and Repairing DNA Initial pairing errors in nucleotide placement may occur as often as 1 per 100,000 base pairs. The amazing accuracy of DNA replication (one error in 10 billion nucleotides) is achieved because DNA polymerases check each newly added nucleotide against its template and remove incorrect nucleotides. Other enzymes also fix incorrectly paired nucleotides, a process called **mismatch repair**.

A large number of different DNA repair enzymes monitor and repair damaged DNA. In **nucleotide excision repair**, the damaged strand is cut out by a **nuclease** and the gap is correctly filled through the action of a DNA polymerase and ligase. In skin cells, nucleotide excision repair frequently corrects *thymine dimers* caused by ultraviolet rays in sunlight.

Evolutionary Significance of Altered DNA Nucleotides Mutations result when uncorrected mismatched nucleotides are replicated and the change is passed on to daughter cells (or to offspring, if the mutation is in a gamete). Although usually harmful, these permanent genetic changes provide the variation on which natural selection operates.

Replicating the Ends of DNA Molecules Because DNA polymerase cannot attach nucleotides to the 5′ end of a growing DNA strand, repeated replications cause a progressive shortening of linear DNA molecules. Multiple repetitions of a short nucleotide sequence at the ends of chromosomes, called **telomeres**, protect an organism's genes from being eroded during successive DNA replications.

The unavoidable shortening of telomeres may limit cell division in somatic cells. In eukaryotic germ cells, however, the enzyme *telomerase* lengthens telomeres. Some somatic cancer cells and "immortal" strains of cultured cells produce telomerase and are thus capable of unregulated cell division.

INTERACTIVE QUESTION 16.6

Draw the last Okazaki fragment being formed on the lagging strand of a linear DNA molecule. Indicate how this results in a shortening of the end of the DNA molecule.

INTERACTIVE QUESTION 16.7

List the successive levels of packing in a metaphase chromosome.

16.3 A chromosome consists of a DNA molecule packed together with proteins

A bacterial cell's double-stranded, circular DNA molecule (associated with a small amount of protein) is supercoiled and tightly packed into a region of the cell called the nucleoid.

In eukaryotes, each chromosome consists of a single, extremely long DNA double helix precisely associated with a large amount of protein, forming a complex called **chromatin**. This DNA fits into the nucleus through a multilevel system of coiling and folding.

Histones are small, positively charged proteins that bind tightly to the negatively charged DNA. Unfolded chromatin appears as a string of beads, each bead a **nucleosome** consisting of the DNA helix wound around a protein core of four pairs of the main histone types. The "string" between beads is called *linker DNA*. The amino end (*histone tail*) of each of the eight histones extends outward. Also called the *10-nm fiber* because of its diameter, nucleosomes are the basic unit of DNA packing. A fifth histone, H1, attaches to the DNA near the bead.

The *30-nm fiber* is a tightly coiled fiber of nucleosomes organized with the aid of histone H1, histone tails, and linker DNA. The *looped domain* consists of loops of the 30-nm chromatin fiber attached to a protein scaffold, forming a *300-nm fiber.* In a metaphase chromosome, looped domains coil and fold, further compacting the chromatin into chromatids that are 700 nm wide and visible with a light microscope.

Some DNA packing is evident in interphase chromosomes, and a chromosome's looped domains appear to be attached to specific locations of the nuclear lamina inside the nuclear envelope. A technique for "painting" individual chromosomes different colors has shown that each chromosome occupies a discrete area in the interphase nucleus.

Certain regions of chromatin, called **heterochromatin**, are in a highly condensed state during interphase. The more open form of interphase chromatin, called **euchromatin**, is available for the transcription of genes.

Word Roots

helic- = a spiral (*helicase:* an enzyme that untwists the double helix of DNA at replication forks, separating the two strands)

liga- = bound or tied (*DNA ligase:* a linking enzyme essential for DNA replication; catalyzes the bonding of the 3′ end of one DNA fragment to the 5′ end of another DNA fragment)

-phage = to eat (*bacteriophage:* a virus that infects bacteria)

telos- = an end (*telomere:* the repetitive DNA at the end of a eukaryotic chromosome that protects the organism's genes from being eroded during successive rounds of replication)

trans- = across (*transformation:* a change in genotype and phenotype due to the assimilation of external DNA by a cell)

Structure Your Knowledge

1. Summarize the evidence and techniques Watson and Crick used to deduce the double-helix structure of DNA.
2. Review your understanding of DNA replication by describing the key enzymes and proteins (in the order of their functioning) that direct replication.

Test Your Knowledge

MULTIPLE CHOICE: *Choose the one best answer.*

1. One of the reasons most scientists thought proteins were the carriers of genetic information was that
   a. the protein content of duplicating cells always doubles prior to division.
   b. proteins are much more complex and heterogeneous than nucleic acids.
   c. early experimental evidence pointed to proteins as the hereditary material.
   d. proteins are found in DNA.

2. Transformation involves
   a. the uptake of external genetic material, often from one bacterial strain to another.
   b. the creation of a strand of RNA from a DNA molecule.
   c. the infection of bacterial cells by phage.
   d. the type of semiconservative replication shown by DNA.

3. The DNA of an organism has thymine as 20% of its bases. What percentage of its bases would be guanine?
   a. 20% c. 40%
   b. 30% d. 60%

4. In his work with pneumonia-causing bacteria, Griffith found that
   a. DNA was the transforming agent.
   b. the pathogenic and harmless strains mated.
   c. heat-killed harmless cells could cause pneumonia when mixed with heat-killed pathogenic cells.
   d. a substance was transferred to harmless cells to transform them into pathogenic cells.

5. T2 phage is grown in *E. coli* with radioactive phosphorus and then allowed to infect other *E. coli*. The culture is blended to separate the viral coats from the bacterial cells and then centrifuged. Which of the following statements best describes the expected results of such an experiment?
   a. Both viral and bacterial DNA molecules are labeled; radioactivity is found in the liquid above the pellet.
   b. Viral DNA is labeled; radioactivity is found in the pellet.
   c. Viral proteins are labeled; radioactivity is found in the liquid but not in the pellet.
   d. Both viral and bacterial proteins are labeled; radioactivity is present in both the liquid and the pellet.

6. Watson and Crick concluded that each base could not pair with itself because
   a. there would not be room for the helix to make a full turn every 3.4 nm.
   b. the uniform width of 2 nm would not permit two purines or two pyrimidines to pair together.
   c. identical bases could not hydrogen-bond together.
   d. they would be on antiparallel strands.

7. In their classic experiment, Meselson and Stahl
   a. provided evidence for the semiconservative model of DNA replication.
   b. were able to separate phage protein coats from *E. coli* by using a blender.
   c. found that DNA labeled with ^{15}N was of intermediate density.
   d. found that DNA composition was species specific.

8. The joining of nucleotides in the polymerization of DNA requires energy from
   a. the hydrolysis of the terminal phosphate group of ATP.
   b. RNA nucleotides.
   c. the phosphate groups of the sugar-phosphate backbone.
   d. the hydrolysis of the pyrophosphates removed from the joining nucleotides.

9. The continuous elongation of a new DNA strand along one of the template strands of DNA
   a. requires the action of DNA ligase as well as polymerase.
   b. occurs because DNA ligase can only elongate in the 5′ → 3′ direction.
   c. occurs on the leading strand.
   d. All of the above are correct.

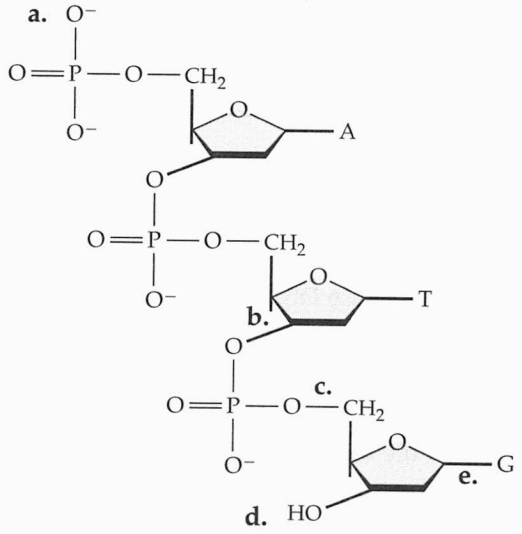
10. Which of the following statements about DNA polymerase is *incorrect*?
    a. It forms the bonds between complementary base pairs.
    b. It is able to proofread and correct errors in base pairing.
    c. It is unable to initiate synthesis; it requires an RNA primer.
    d. It only works in the 5′ → 3′ direction.

11. Thymine dimers—covalent links between adjacent thymine bases in DNA—may be induced by UV light. Which of the following enzymes is *not* involved in the repair of these dimers?
    a. excision enzymes (nucleases)
    b. DNA polymerase
    c. ligase
    d. primase

12. How does DNA synthesis along the lagging strand differ from that on the leading strand?
    a. Nucleotides are added to the 5′ end instead of the 3′ end.
    b. Ligase is the enzyme that polymerizes DNA on the lagging strand.
    c. An RNA primer is needed on the lagging strand but not on the leading strand.
    d. Okazaki fragments, which each grow 5′ → 3′, must be joined along the lagging strand.

13. Which of the following enzymes or proteins is paired with an incorrect or inaccurate function?
    a. Helicase—unwinds and separates parental double helix
    b. Telomerase—adds telomere repetitions to ends of chromosomes
    c. Single-strand binding protein—holds strands of unwound DNA apart and straight
    d. Primase—forms DNA primer to start replication

Use the following diagram to answer questions 14 through 17.



14. Which letter indicates the 5′ end of this single DNA strand?
    a. b. c. d. e.

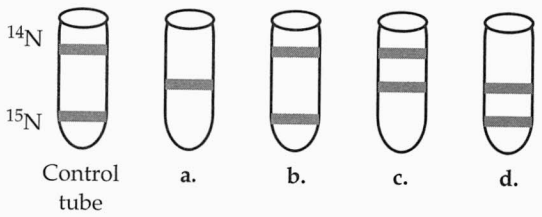
15. At which letter would the next nucleotide be added?
    a. b. c. d. e.

16. Which letter indicates a phosphodiester bond formed by DNA polymerase?
    a. b. c. d. e.

17. The base sequence of the DNA strand made from this template would be (from bottom to top)
    a. ATG c. TAC.
    b. CAT. d. UAC.

18. Which of the following statements about telomeres is correct?
    a. They are ever-shortening tips of chromosomes that may signal cells to stop dividing at maturity.
    b. They are highly repetitive sequences at the tips of chromosomes that protect the leading strand during replication.
    c. They are enzymes in germ cells that allow these cells to undergo repeated divisions.
    d. All of the above are correct.

19. You are trying to test your hypothesis that DNA replication is *conservative*— that is, that the parental strands separate, newly made complementary strands join together to make a new DNA molecule, and the parental strands then rejoin. You take a sample of *E. coli* grown in a medium containing only heavy nitrogen (^{15}N) and transfer it to a medium containing light nitrogen (^{14}N). After allowing time for only one DNA replication, you centrifuge a sample and compare the density band(s) formed to the bands formed from bacteria grown on either normal ^{14}N or ^{15}N medium. Which band location would support your hypothesis of *conservative* DNA replication?



Bands after one replication on ^{14}N

20. Given the experimental procedure explained in question 19, which centrifuge tube (obtained after one DNA replication) would represent the band distribution indicating that DNA replication is *semiconservative*?

21. If the following molecules or structures were put in order from smallest to largest, which structure would be at the bottom of that size range?
    a. looped domain
    b. histone
    c. nucleosome
    d. 30-nm fiber

22. Biologists have learned from the technique of "painting chromosomes" with different-colored molecular tags that
    a. the two homologs of a pair of chromosomes differ enough that they stain different colors.
    b. chromosome packing occurs only as the cell prepares for mitosis or meiosis.
    c. heterochromatin is concentrated at the tips and centromeres of chromosomes.
    d. in the interphase nucleus, each chromosome appears to occupy a specific area.